DETAILED ACTION

Amendment Entry

1. Applicant's amendment, filed 12/20/2011, is acknowledged and has been entered. Claims 29, 31, 47, 57, and 60 were amended. Claim 59 was canceled. Accordingly, claims 29, 31-38, 47, 57-58, and 60 are currently pending and subject to examination below in light of the elected species of amino acids 32-48 of canine proBNP (SEQ ID NO:3) as the species of proBNP epitope.

Manner of Making Amendments under 37 CFR 1.121

2. In the interest of expediting prosecution, Applicant's submission has been accepted. However, Applicant is reminded of the proper format for amendments to the claims. Claim 59 is listed with the status of "Canceled" and Applicant's reply also states that the claim has been canceled (section A). However, the claim set also includes text for this claim. No claim text should be presented for any claim in the claim listing with the status of "canceled". See MPEP 714.

Rejections Withdrawn

3. The Declaration under 37 CFR 1.132 filed on 12/20/2011 by Dr. Farace ("Second Farace Declaration") is sufficient to overcome the rejection of claims 29, 31-38, 47, 57-58, and 60 based upon 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement.

Specifically, the Second Farace Declaration states that an antibody directed against the sequence KDAVSELQAEQLALEPL (i.e., SEQ ID NO:3) successfully recognized and bound to canine plasma proBNP (item 4). The Second Farace Declaration also points to the First Farace

Declaration and states that the peptide referred to as "amino acids 25-41" of canine proBNP has the amino acid sequence KDAVSELQAEQLALEPL (i.e., SEQ ID NO:3; see Second Farace Declaration, item 5). The Declarations by Dr. Farace therefore show that the claimed invention works as intended, in that an antibody that binds to an epitope within SEQ ID NO:3 is capable of binding to canine proBNP in plasma.

It is noted that item 2 of the Second Farace Declaration states that "these antibodies successfully recognize and bind to canine proBNP that is present in a **feline** plasma sample" (emphasis added). However, since items 3 and 4 refer to "canine plasma ", this reference to a "feline plasma sample" in item 2 is understood to be an error of "canine plasma sample".

Priority

4. Acknowledgment is made of the present application as a proper National Stage (371) entry of PCT Application No. PCT/EP05/54446, filed 9/8/2005, which claims foreign priority under 35 U.S.C. 119(a)-(d) to Application No. A 1505/2004, filed on 9/8/2004 in Austria.

Claim Rejections - 35 USC § 112

5. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

6. Claims 29, 31-38, 47, 57-58, and 60 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled

in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

New Matter

7. Claim 29, as amended in the instant reply, refers to the determination of canine proBNP or "Nt-proBNP fragments thereof". This reference to **Nt-proBNP fragments** in the plural suggests that there may be multiple different Nt-proBNP fragments which are determined by the claimed methods.

The specification refers to canine Nt-proBNP in the singular (published application at [0026], [0074]. The specification also refers to "the amino acid sequence of...canine Nt-proBNP [0074]. While the specification suggests the determination of "proBNP or their fragments", Nt-proBNP is mentioned as an exemplary fragment [0026]. The specification therefore uses the term "Nt-proBNP" in such a way as to convey a single molecule or fragment of proBNP, and provides no suggestion that there could be multiple different Nt-proBNP fragments. Similarly, the term "NT-proBNP" was used in the prior art to refer to a specific molecule (see, e.g., Karl et al. as discussed in detail below). There is no indication that the term "NT-proBNP" was used in the prior art to refer to a genus of fragments. The claims therefore introduce new matter as they suggest that there may be multiple "Nt-proBNP fragments" that are determined by the method, a concept not introduced in the specification claims as originally filed.

Written Description

8. The nature of the invention relates to an immunoassay method for detecting canine proBNP or "NT-proBNP fragments thereof" in a canine blood sample. The methods uses an antibody that binds an epitope within the amino terminus of canine proBNP, in particular the elected species of an epitope within SEQ ID NO:3 (see especially claims 29, 31, 47 and 57).

Applicant has provided evidentiary testimony that, as of 2004, it was unknown how many forms of BNP existed in tissue and in blood and that further, it was unclear which amino acid regions of the various forms of BNP could be detected using antibodies. In particular, Applicant declares that it was not possible at the time of invention to predict that any circulating form of BNP would also present and be able to be detected using antibodies in dogs. Applicant further declares that it was unclear at the time of invention which of the various BNP forms would be useful as markers of pathophysiologic processes. See the Declaration of Dr. Boswood filed 5/18/2010, in particular at items 4-7, 14-15, and 18-20.

For example, Dr. Boswood declares that as of 2004 it was not possible to predict that an antibody against a particular amino acid region of proBNP could detect proBNP or a particular N-terminal fragment thereof (item 5). In items 19-20, Dr. Boswood further declares that:

> More specifically, the following were neither known nor could be predicted: (1) how many forms of BNP were present in the tissues and blood of humans and other species; (2) which forms of circulating BNP would be predominant in species such as cats and dogs; (3) whether any forms of circulating BNP would be stable enough to be detected by antibodies in cats or dogs; and (4) which forms of BNP would be useful as markers of pathophysiologic processes.
>
> For the foregoing reasons, as of September 8, 2004, it was unknown whether circulating proBNP could be detected in dogs or cats. Furthermore, it was not possible to predict that antibodies directed against a particular amino acid region of dog proBNP could detect circulating proBNP in dogs. Likewise, it was not possible to predict that antibodies directed against a particular amino acid region of cat proBNP could detect circulating proBNP in cats.

Dr. Boswood further declares that that "it was not possible to predict that an antibody against a particular amino acid region of proBNP could detect proBNP or a particular N-terminal fragment of proBNP" and similarly that "it was unclear which amino acid regions of the various forms of BNP could be detected using antibodies" (see items 5 and 7, respectively).

Such statements by Applicant indicate that at the time of invention, it was not possible to predict whether fragments of canine proBNP containing exist in canine blood, and that in particular, it was not possible to predict whether fragments containing epitopes found within SEQ ID NO:3 of canine proBNP would exist in these samples. Further, Applicant's remarks indicate that it was not possible to predict whether such fragments would provide informative clinical information about the presence of disease.

If the existence of canine proBNP or fragments thereof in canine blood could indeed not be predicted as argued by Applicant, this means that one of ordinary skill in the art would not envisage possession of the claimed methods without verification that such species actually exist and are correlated with disease.

The instant specification discloses experiments in which antibodies raised against amino acids 25-41 and 74-86 of canine proBNP were used to analyze samples from healthy and sick dogs. See Example 3 and Figure 3A in particular. However, the instant specification fails to disclose experiments in which antibodies were raised against SEQ ID NO:3 of canine proBNP. Consequently, the data presented in the specification do not verify the existence of canine proBNP or fragments thereof that contain SEQ ID NO:3.

In summary, Applicant has taken the position in the instant Reply that it was not possible to predict *a priori* whether antibodies binding to particular region of canine proBNP could be used to detect proBNP in blood. If adopted, such arguments and evidence mean that it would not be possible to predict whether an antibody binding to an epitope within SEQ ID NO:3 of canine proBNP could be used to detect canine proBNP or fragments thereof in the blood of dogs. Yet the instant specification adds nothing further to this inquiry, as such an antibody was not actually made.

Similarly, if it is true as Applicants argue that the existence of proBNP or fragments thereof that are detectable via the claimed antibody could not have been predicted at the time of invention, and further that it could not be predicted whether such fragments were stable enough to be detected, then this means that actual data or other means of verifying their existence would be necessary in order to envisage possession of the claimed invention. In addition, if Applicant's

evidence and arguments that it was not possible to predict which forms of BNP would be useful as markers of pathophysiological processes are adopted, this means that data regarding BNP forms that are detectable by the claimed antibodies and their correlation with cardiac insufficiency would be necessary. Yet the instant specification adds nothing further to this inquiry, as such data documenting the existence of the BNP species at issue and their correlation with disease are also lacking in the instant specification.

9. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

10. Claims 29, 31-33, 37-38, 47, 57-58, and 60 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

11. Claim 29 refers to the determination of canine proBNP or "Nt-proBNP fragments thereof". This reference to Nt-proBNP fragments in the plural suggests that there may be multiple different Nt-proBNP fragments.

The specification refers to canine Nt-proBNP in the singular (published application at [0026], [0074]. The specification also refers to "the amino acid sequence of...canine Nt-proBNP [0074]. The specification therefore provides no suggestion that there could be multiple different Nt-proBNP fragments. Similarly, there is no indication that the term "NT-proBNP" was used in the prior art to refer to a genus of fragments. As a result, it is unclear what is meant by "Nt-proBNP fragments thereof". The metes and bounds of this term are unclear; it is not known what

molecules other than NT-proBNP *per se* would be encompassed by the recitation of "Nt-proBNP fragments".

Claim Rejections - 35 USC § 103

12. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

13. Claims 29, 31-33, 37-38, 47, and 57-58 are rejected under 35 U.S.C. 103(a) as being unpatentable over MacDonald et al. ("Brain natriuretic peptide concentration in dogs with heart disease and congestive heart failure" J Vet Intern Med. 2003 Mar-Apr;17(2):172-7) in view of Asada et al. (EP 1 016 867 B1, of record) and in light of the evidence of Harlow & Lane ("Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988, pages 23-24 and 76), the Academic Press Dictionary of Science and Technology (definition for the term "polyclonal"; Oxford: Elsevier Science & Technology (1996); retrieved October 22, 2008, from http://www.credoreference.com/entry/3144515/), Janeway et al. (Immunobiology: the Immune System in Health and Disease (1999), Elsevier Science Ltd/Garland Publishing, New York, NY, Fourth Edition, pages 34-35), and Wolfe (Wolfe, S.L., Molecular and Cellular Biology, 1993, pages 790-793).

MacDonald et al. teaches that brain natriuretic peptide (BNP) is a recognized biomarker of cardiac disease and congestive heart failure in humans (the abstract). The authors performed clinical studies on dogs, in order to assess whether BNP is also a biomarker of canine heart

disease. In particular, MacDonald et al. measured canine BNP levels in plasma samples from normal dogs and from dogs with heart disease or heart failure, and observed a significant positive correlation with disease (ibid and pages 174-176, "Discussion"). In addition, BNP levels increased with increasing severity of disease (ibid and pages 173-174, "Results"). MacDonald et al. conclude from these studies that increases in BNP may be used to predict death due to cardiovascular disease in dogs, much like in human medicine (see page 175, last paragraph to page 176, second paragraph).

The teachings of MacDonald et al. indicate that BNP is indicative of disease not only in humans, but also in dogs.

It is noted that MacDonald et al. measured BNP-32 (which is a fragment of proBNP corresponding to the carboxy-terminal 32 amino acids of proBNP which is released upon proteolytic cleavage) by radioimmunoassay (page 173, left column), but do not provide details regarding the measurement.

The teachings of MacDonald et al. differ from the claimed invention in that while the reference determined the concentration of the BNP-32 fragment of canine proBNP by immunoassay, the reference fails to apparently teach the use of an antibody that binds to an epitope within the NT-proBNP region of canine proBNP.

Asada et al. teach that BNP-32 (also referred to in the reference as α-BNP or simply BNP) is first synthesized as the preprohormone prepro-BNP [0002]. This preprohormone includes a signal sequence which is cleaved to give pro-BNP (also referred to in the reference as γ-BNP) (ibid and [0010]). In the case of humans, pro-BNP has a total of 108 amino acids [0011]. Pro-BNP is then further split to produce BNP-32 and BNP(1-76). The latter fragment is also

referred to in the reference as γ-BNP(1-76). BNP-32 is the carboxy-terminal fragment of proBNP, while BNP(1-76) is NT-proBNP or the amino-terminal fragment of proBNP [0007].

Applicant has also argued on the record that it was known in the prior art that canine proBNP is cleaved into NT-proBNP and BNP, and that the NT-proBNP region of canine proBNP was also known (Applicant's arguments of 12/20/2011, see section D).

Asada et al. teach in blood, BNP exists in the form of proBNP or its degradation product, and not in the form of BNP-32 which was previously considered dominant [0008]. Asada et al. further teach that pro-BNP is more stable than BNP-32 in blood (ibid). Due to these facts, Asada et al. concluded that it is indispensable to assay not only BNP-32 but also pro-BNP in order to accurately diagnose cardiac disease.

To accomplish this, Asada et al. teach an immunoassay using a first antibody which is reactive with BNP-32 and a second antibody which is reactive with mammalian prepro-BNP or pro-BNP derivatives but not with BNP-32 [0009], [0012], [0017]. Note that Asada employ the term "derivatives" to encompass pro-BNP itself [0013].

The immunoassay preferably measures the pro-BNP derivative that corresponds to amino acid Nos. 27-134 of SEQ ID NO:1, in the case of human BNP [0019]. This sequence refers to pro-BNP, i.e. after cleavage of the 26-amino acid N-terminal signal sequence from prepro-BNP.

The second antibody used for the assay is preferably specific for amino acids 27-102 of SEQ ID NO:1 in the case of the human sequence [0019]. This sequence corresponds to BNP(1-76), i.e., NT-proBNP or pro-BNP after the carboxy-terminal BNP-32 has been removed. As one example, Asada et al. raised an antibody against amino acid Nos. 27-64 of SEQ ID NO:1 (i.e., amino acids 1-38 of human pro-BNP). See Example 1, in particular at page 5, lines 24-32.

Although the Example of Asada et al. involved antibodies against human BNP sequences, the reference clearly contemplates any mammalian proBNP and also specifically mentions canine proBNP (see [0010] and claim 1).

In light of the teachings of Asada et al., it would have been obvious to one of ordinary skill in the art to modify the teachings of MacDonald et al. so as to detect not only canine BNP-32 but also canine pro-BNP. In particular, the teachings of Asada et al. indicate that contrary to what was previously thought, BNP exists predominantly in the blood not as BNP-32 but as proBNP or other fragments thereof. In addition, Asada et al. taught that proBNP is more stable in blood than BNP-32. Consequently, Asada et al. teach the need to employ an assay that detects not only BNP-32 but also pro-BNP in order to accurately assess cardiac disease.

As such, when assessing cardiac disease in dogs based on BNP levels according to the method of MacDonald et al., one of ordinary skill in the art would have been motivated to employ this known variation by performing an immunoassay that detects not only canine BNP-32 but also canine pro-BNP in order to obtain more accurate clinical results in dogs in the same way that Asada et al. taught in the case of humans.

Put another way, because Asada et al. teach that their immunoassay produces more accurate clinical results than assays for BNP alone, it would have been obvious to adapt this immunoassay in order to detect pro-BNP and BNP in any mammalian species; when taken together with the teachings of MacDonald et al. that BNP was also well established to be biomarker of heart failure in dogs, it would have been obvious to adapt the immunoassay format of Asada et al. to detect canine pro-BNP and BNP for the purpose of clinical assessment of heart failure in dogs.

The particular solution taught by Asada et al. for performing such an improved BNP immunoassay employs two antibodies, one specific for the carboxy-terminal fragment of pro-BNP (BNP-32) and one specific for NT-proBNP, the amino-terminal fragment of pro-BNP (which is BNP(1-76) in the case of the human sequence).

The Examiner notes that both human and canine BNP-32 are peptides of 32 amino acids (Asada et al., [0002]). However, the precursor pro-BNP sequences vary slightly depending on species (Asada et al., [0010]). This is why when Asada et al. refer to particular amino acid sequences or residues, the species is also specified (e.g., "In case of human [pro-BNP], it is pro-BNP of 108 amino acids" [0011]).

Therefore, when Asada et al. teach that the second antibody is preferably specific to BNP(1-76) or amino acids 27-102 of SEQ ID NO:1, one of ordinary skill in the art would readily understand that this numbering is referring to the amino-terminal fragment of *human* prepro-BNP (i.e., amino acids 1-76 of proBNP after removal of the 26-residue signal sequence). However, because Asada et al. clearly contemplates *any* mammalian proBNP and also specifically mentions canine proBNP (see [0010] and claim 1), it would have been obvious when performing assays for dog pro-BNP to employ a second antibody that is specific for the amino-terminal fragment of canine pro-BNP. Furthermore, Asada et al. also indicate that like human proBNP, canine proBNP is also cleaved into amino-terminal and carboxy-terminal fragments, although this occurs at Arg 100 rather than Arg 102 due to slight differences in the sequences between species [0010].

Applicant has also argued on the record that it was known in the prior art that canine proBNP is cleaved into NT-proBNP and BNP, and that the NT-proBNP region of canine proBNP was also known (Applicant's arguments of 12/20/2011, see section D).

Therefore, it would have been obvious to adapt the two-antibody assay of Asada et al. for detection of canine pro-BNP and BNP by employing a first canine BNP-32-specific antibody together with a second antibody specific for canine NT-proBNP, the amino-terminal fragment of canine proBNP which is released upon cleavage at Arg 102 of canine proBNP. One would be motivated to use two antibodies specific for both the amino-terminal and carboxy-terminal fragments of proBNP in this manner in order to detect not only BNP-32 but also proBNP and its derivatives, which leads to more accurate clinical results as taught by Asada et al.

The combination of MacDonald et al. and Asada et al. therefore suggest the use of an antibody binding to canine NT-proBNP, the amino-terminal fragment of canine proBNP (i.e., the canine counterpart of BNP(1-76) in humans).

In addition, no evidence of criticality for the currently claimed range is apparent. Because the teachings of Asada et al. indicate that the region to which antibodies bind on preproBNP influence what fragments of this molecule may be detected, it would have been obvious to arrive at the claimed invention out of the course of routine optimization.

Similarly, with respect to claims 31, 47, and 57-58, although the references do not specifically teach the recited epitope consisting of SEQ ID NO:3, absent evidence of criticality it would have been obvious to arrive at the claimed invention out of the course of routine optimization.

It is also noted that Asada et al. teach that the antibodies used to determine proBNP may be either monoclonal or polyclonal [0018].

Polyclonal antiserum was well known in the art to comprise a mixture of antibodies of different specificities directed toward multiple antigenic determinants present on a particular antigen. See the Academic Press Dictionary of Science and Technology, which defines a polyclonal antibody as a population of heterogeneous antibodies derived from multiple clones, each of which is specific for one of a number of determinants found on an antigen. See also Janeway et al., which provides evidence that antibodies in serum (i.e., antisera) are polyclonal in nature, containing many different antibody molecules that bind to an antigen in many different ways (see p. 34-35, especially at p. 35, the second full paragraph, and Figure 2.1).

It was also well known in the art at the time of the invention that antibodies do not contact the entire surface of their target antigen but rather bind relatively small regions or "epitopes" within said antigen. See Harlow & Lane at pages 23-24, the section entitled "The region of an antigen that binds to an antibody is called an epitope". Harlow et al. also provide evidence that peptide epitopes recognized by antibodies are generally only six amino acids in length, with some researchers reporting epitopes of even smaller size that can be successfully bound by an antibody (see page 76, the first sentence of the section titled "Size of the Peptide"). Similarly, Wolfe discloses that the size of an epitope bound by an antibody is between 3 to 16 amino acids in length (see particularly the bottom of the left column of page 791).

In light of the evidence of Harlow et al. and Wolfe, it is apparent that the recited amino acid sequences possess multiple epitopes available for antibody binding.

Furthermore, in light of the evidence of the Academic Press Dictionary of Science and Technology and Janeway et al., it is apparent that the polyclonal antibodies of Asada et al. would necessarily constitute a homogeneous population of antibodies that bind to the immunogens in many different ways, i.e. to different epitopes within this peptide.

As such, an especially absent evidence to the contrary, it appears that the polyclonal antibodies specific to the amino-terminal fragment of canine proBNP (canine NT-proBNP) as suggested by the combination of MacDonald et al. and Asada et al. would necessarily include antibodies capable of binding to proBNP at an epitope within SEQ ID NO:3, given that this sequence shares multiple epitopes in common with the canine NT-proBNP.

Therefore, it would also have been obvious to arrive at the claimed invention of claims 29, 31-38, 47 and 57 by employing antibodies as above that are polyclonal, as taught by Asada et al. As a result of the nature of such antibodies as well as the relationship of the recited sequences to the amino-terminal fragment of canine proBNP, it appears that the resulting polyclonal antibodies would necessarily possess the recited binding characteristics.

With respect to claim 32, Wolfe (discussed above) provides evidence that antibody epitopes are 3 to 16 amino acids in length. Therefore, antibodies to canine proBNP according to the method of MacDonald et al. and Asada et al. would necessarily possess the recited characteristics.

With respect to claim 37, in the immunoassay of Asada et al., at least one of the first and second antibodies may be immobilized on a solid support [0021]. Therefore, when performing the method of MacDonald et al. using two pro-BNP-specific antibodies as taught by Asada et al.,

it would have been obvious to employ this solid phase-based assay format suggested by Asada et al.

With respect to claim 38, the immunoassay of MacDonald et al. and Asada et al. involves antibody binding and would therefore be considered an "immune binding assay".

With respect to claim 58, Asada et al. teach as above that the antibodies may be either monoclonal or polyclonal [0018].

14. Claims 34-36 are rejected under 35 U.S.C. 103(a) as being unpatentable over MacDonald et al. in view of Asada et al. and in light of the evidence of Harlow & Lane, the Academic Press Dictionary of Science and Technology, Janeway et al., and Wolfe as applied to claim 29 above, and further in view of Harlow & Lane ("Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988, pages 319, 321-323, 342-345, 353, 561, 563, 574, and 591-593; hereinafter, "Harlow & Lane **2**"). See Applicant's Information Disclosure statement filed 3/27/2009.

MacDonald et al. and Asada et al. are as discussed in detail above. MacDonald et al. teaches radiolabeling (page 173, left column) but does not provide further details. Asada et al. exemplifies directly labeling one of the antibodies used for the two-antibody assay (Example 1), and therefore fails to specifically teach the use of at least one further antibody that binds to the antibody discussed in detail above.

Harlow & Lane **2** teach that an antibody can be directly labeled or alternatively used with a labeled secondary reagent that will specifically recognize the antibody (see entire selection, especially at pages 321, 561 and 563). Such a secondary reagent may be (for example) a labeled

anti-immunoglobulin antibody specific for the first antibody (see in particular pages 321-323, 345, 574 and 591-593). Detection labels may be, e.g. iodine, fluorochromes, enzymes such as peroxidase, or biotin (p. 342-344, 353, 561, 578-580, 591-593, and 320-323). The choice of direct versus indirect detection depends on the circumstances of the experiment; however, the use of directly labeled antibodies is less sensitive than indirect methods (page 321). In addition, direct labeling requires a new labeling step for every antibody to be studied. In contrast, indirect methods offer the advantages of widely available labeled reagents, which are commercially available and which can be used to detect a large range of antigens. Finally, with indirect labeling the primary antibody is not modified, so potential loss of activity is avoided. Consequently, for the majority of applications indirect methods are the most useful.

Therefore, it would have been further obvious to add a further secondary antibody specific for the proBNP-specific antibody in labeled form when performing the method of MacDonald et al. and Asada et al. In particular, it would have been obvious to substitute indirect labeling in this manner for the direct labeling methods exemplified by Asada et al. because Harlow & Lane **2** taught for most applications, indirect methods are the most useful and offer numerous advantages over direct labeling.

With respect to claim 36, both MacDonald et al. and Asada et al. teach the use of radiolabels (MacDonald et al. at page 173, left column; radiolabeled iodine is taught by Asada et al. at page 5). Asada also teaches enzymes, fluorescent substances, particles, etc. Similarly, Harlow & Lane that detection labels may be, e.g. iodine, fluorochromes, enzymes such as peroxidase, etc.

Therefore, when employing indirect labeling to label the secondary antibody as discussed above, it would have been further obvious to employ such known labels for their known purpose.

15. Claim 60 is rejected under 35 U.S.C. 103(a) as being unpatentable over MacDonald et al. in view of Asada et al. and in light of the evidence of Harlow & Lane, the Academic Press Dictionary of Science and Technology, Janeway et al., and Wolfe as applied to claim 29 above, and further in view of Hrubec et al. ("Plasma Versus Serum: Specific Differences in Biochemical Analyte Values" Journal of Avian Medicine and Surgery 16(2):101-105, 2002).

The references are as discussed in detail above, which fail to specifically teach analysis of **serum** samples. In particular, MacDonald et al. and Asada et al. suggest plasma samples but not serum samples.

Hrubec et al. teach that biochemical analysis can be conducted using either plasma or serum, and that these are similar sample types (page 101, right column). The reference teaches that the different sample types have different handling requirements in that plasma samples are kept on ice and centrifuged immediately, while serum samples can be allowed to clot at room temperature before centrifuging (the abstract and page 103, "Discussion").

Therefore, one skilled in the art would find it obvious to detect canine pro-BNP in serum rather than in plasma for convenience, in order to avoid having to immediately cool and centrifuge the collected sample. One would have a reasonable expectation of success given that Hrubec et al. teach that serum and plasma are similar samples differing only in that plasma contains clotting factors and an anticoagulant that are not present in serum. Thus, since natriuretic peptides such as proBNP are neither clotting factors nor anticoagulants, one skilled in

the art would have a reasonable expectation of success in detecting canine pro-BNP in serum as well as plasma.

16. Claims 29, 31-38, 47, 57-58, and 60 are rejected under 35 U.S.C. 103(a) as being unpatentable over MacDonald et al. ("Brain natriuretic peptide concentration in dogs with heart disease and congestive heart failure" J Vet Intern Med. 2003 Mar-Apr;17(2):172-7) in view of Karl et al. (U.S. 2007/0059767 A1) and Liu et al. ("Cloning and characterization of feline brain natriuretic peptide" Gene 292 (2002) 183–190).

MacDonald et al. teaches that brain natriuretic peptide (BNP) is a recognized biomarker of cardiac disease and congestive heart failure in humans (the abstract). The authors performed clinical studies on dogs, in order to assess whether BNP is also a biomarker of canine heart disease. In particular, MacDonald et al. measured canine BNP levels in plasma samples from normal dogs and from dogs with heart disease or heart failure, and observed a significant positive correlation with disease (ibid and pages 174-176, "Discussion"). In addition, BNP levels increased with increasing severity of disease (ibid and pages 173-174, "Results"). MacDonald et al. conclude from these studies that increases in BNP may be used to predict death due to cardiovascular disease in dogs, much like in human medicine (see page 175, last paragraph to page 176, second paragraph). The teachings of MacDonald et al. indicate a nexus between BNP and cardiac disease not only in humans, but also in dogs.

It is noted that MacDonald et al. measured BNP-32 (which is a fragment of proBNP corresponding to the carboxy-terminal 32 amino acids of proBNP which is released upon

proteolytic cleavage) by radioimmunoassay (page 173, left column), but do not provide further details regarding the measurement.

The teachings of MacDonald et al. differ from the claimed invention in that while the reference determined the concentration of the BNP-32 fragment of canine proBNP by immunoassay, the reference fails to apparently teach the use of an antibody that binds to an epitope within SEQ ID NO:3.

Karl et al. discuss how human BNP is expressed as the 108-residue precursor proBNP, which is cleaved into N-terminal proBNP (NT-proBNP; amino acids 1-76 of proBNP) and the BNP (amino acids 77-108 of proBNP, also known in the art as BNP-32). See [0007].

Karl et al. teach that because BNP itself is not very stable, its use as a diagnostic marker is limited [0006]. As an alternative to BNP, Karl et al. teach determining NT-proBNP in a sample using two antibodies that detect different epitopes of this protein fragment, such that they are capable of simultaneously binding (the abstract; [0001]; [0011]-[0025], [0046]; and the claims). The antibodies specifically bind epitopes within the 76-amino acid NT-proBNP molecule, preferably in the amino acid region from 10 to 66, and particularly preferred in the region 10 to 50 or 10 to 38 [0044]. The antibodies may be either polyclonal or monoclonal [0025], and may be raised using recombinant NT-proBNP as an immunogen [0043].

In the Examples, Karl et al. raised both polyclonal and monoclonal antibodies against recombinant human NT-proBNP and subsequently screened them to determine the most reactive epitopes. Antibodies which were reactive with amino acids 30-38 of proBNP (ELQVEQTSL; SEQ ID NO:8) were successfully produced in this manner (Example 2, see especially at [0062];

and Example 3, see especially Table 2, monoclonal antibody 13.1.18; and Table 3, polyclonal antibody S-9212).

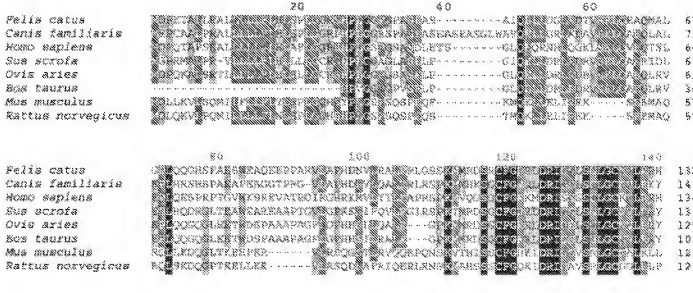
The polyclonal antibody against amino acids 30-38 of human NT-proBNP was used together with a second polyclonal antibody against amino acids 1-21 of this molecule in an immunoassay for determination of NT-proBNP (Examples 4-6). Such assays can be used diagnostically to differentiate between healthy and heart failure patients (Example 6 and claims 28-39).

In summary, Karl et al. teach that like BNP, NT-proBNP is also a diagnostic marker in heart failure, but that NT-proBNP is more stable than BNP. When taken together with the teachings of MacDonald et al. (in which BNP was assessed in the context of canine heart failure), it would have been obvious to one of ordinary skill in the art to detect canine NT-proBNP instead of canine BNP in plasma in the method of MacDonald et al. One would be motivated to do this because Karl et al. taught that the former was known to be a more stable biomarker; as such, assessing changes in the levels of NT-proBNP in the disease state would be less subject to biomarker degradation which would be expected to adversely affect measurements. In addition, patient samples could be stored for longer periods of time before being assayed.

In carrying out assays for canine NT-proBNP, it would have been further obvious to follow the immunoassay strategy set forth in Karl et al., which directs the skilled artisan to employ two antibodies specific for the amino-terminal fragment of pro-BNP or NT-proBNP.

As discussed above, Karl et al. suggests antibodies that recognize amino acids 10-66, 10-50, or 10-38 of the 76-residue human NT-proBNP molecule; and exemplifies antibodies that recognize amino acids 30-38.

Liu et al. teach that the nucleotide and amino acid sequences of BNP have been identified for several mammalian species, including human and dog (page 188, right column). Liu et al. teach that sequence similarity of BNP genes provides strong evidence of related function in mammalian species abstract and page 188, right column, first full paragraph). In Figure 3, the full-length sequences of preproBNP from dog, human, and other species are aligned to show similarities among species:



In their publication, Liu et al. report the newly identified cat BNP sequence and discuss how this information now allows for antibodies to be generated using synthesized antigenic

peptides for immunological assay development, and in particular for clinical applications (paragraph bridging pages 188-189).

This indicates that with knowledge of a protein's amino acid sequence, antibodies can be readily produced against the protein in order to allow its detection by clinical immunoassay. While the cat BNP sequence was newly reported by Liu et al., the sequence for canine preproBNP was previously known in the art (Liu et al., page 183, right column; and Figure 3). Liu et al. also teach that antibodies to BNP were known in the art to be species-specific (page 183, last column).

It is noted that the above sequences of Liu et al. are for preproBNP, and include the N-terminal signal peptide, which is 26 amino acids in length in the case of the feline, canine, and human sequences (see Liu et al., abstract).

Based on the detailed sequence information known in the prior art about the human and canine proBNP molecules (as taught by Liu et al.), one of ordinary skill in the art would have found it obvious to design and raise antibodies against canine NT-proBNP. For example, it would have been obvious to produce canine NT-proBNP in recombinant form as was done by Karl et al. for human BNP, and to raise antibodies against this molecule.

In particular, based on the teachings of Karl et al. that antibodies against residues 30-38 human proBNP can successfully be used to detect human NT-proBNP in clinical assays, one of ordinary skill in the art would have found it obvious to raise antibodies that bind within the corresponding regions of canine NT-proBNP. One would be motivated to combine the reference teachings in this manner because known work in one field of endeavor may prompt variations in another. In the instant case, one or ordinary skill in the art seeking to design antibodies to canine

NT-proBNP would reasonably consider analogous art pertaining to antibodies for the human homolog.

Although the canine and human proBNP sequences do not align exactly, differing in length, the teachings of Liu et al. provide detailed information about how the amino acids in the sequence of human proBNP correspond to those in canine proBNP. Examining the sequence alignment of Liu et al. above, it can be readily seen that amino acids 30-38 of human proBNP (ELQVEQTSL; SEQ ID NO:8 in Karl et al.) correspond to amino acids 37-45 of canine proBNP (subtracting the 26-amino acid N-terminal signal sequence):

30-38 (human): ELQVEQTSL

37-45 (canine): ELQAEQLAL

This canine amino acid sequence **lies entirely within** the sequence depicted SEQ ID NO:3 in the instant specification (Figure 1B).

Therefore, when producing antibodies against the canine sequence analogous to amino acids 30-38 of human proBNP, it would necessarily follow that the resulting antibodies would be specific for an epitope within SEQ ID NO:3 of canine proBNP.

In addition, based on the findings of Karl et al. that amino acids 30-38 of human proBNP (ELQVEQTSL) was one of two epitopes which showed the strongest reaction with the polyclonal antibodies they elicited [0084], as well as the strong sequence and structural similarity between human and canine proBNP taught by Liu et al., it would also be reasonably expected that polyclonal antibodies raised against canine NT-proBNP would also be reactive with the canine NT-proBNP epitope corresponding to amino acids 30-38 of human NT-proBNP.

As such, armed with the knowledge that amino acids 30-38 of human NT-proBNP are highly immunogenic, as well as the detailed sequence information available that these amino acids correspond to amino acids 37-45 of canine NT-proBNP and that these amino acids are largely conserved across the two species, it would have been obvious to one of ordinary skill in the art to target these analogous amino acids in canine NT-proBNP as they would have been reasonably expected to be immunogenic.

Further, when making an antibody against amino acids 37-45 of canine NT-proBNP, the resulting antibody would necessarily bind to amino acids 32-48 of this peptide (i.e., SEQ ID NO:3) since the former sequence lies entirely within the latter.

In view of the knowledge in the art of the field of human proBNP field as taught by Karl et al., and when taken together with the detailed sequence information for both human and canine proBNP available in the art (as taught by Liu et al.), the Examiner finds that the differences between the claimed invention and the prior art were encompassed in known variations.

While the above analysis focuses on the elected species of an antibody binding to an epitope within amino acids 32-48, the examiner notes that claim 29 is not limited to such an epitope but is directed to the broader range of amino acids 20-86 of proBNP. However, it is briefly noted that this broader range is also obvious in view of the reference teachings.

In particular, from the teachings of Karl et al. and Liu et al. as discussed in detail above, one would be motivated to detect canine NT-proBNP (i.e., the N-terminal portion of proBNP). The range suggested by the prior art also corresponds to the N-terminal portion of proBNP and therefore overlaps the currently claimed range, such that a *prima facie* case of obviousness

exists. As such, it would also have been obvious to arrive at the claimed invention through routine optimization, by employing antibodies directed against epitopes within the N-terminal fragment of canine proBNP as suggested by Karl et al.

With respect to claim 32, Karl et al. teach that usually an epitope is clearly defined by 6 to 8 amino acids [0016]. In light of this evidence, it is presumed that the antibodies raised against canine NT-proBNP would possess the recited characteristics.

With respect to claims 33 and 58, Karl et al. teach that the antibodies may be either monoclonal or polyclonal [0025].

With respect to claims 34-36, Karl et al. teach that the antibody may be labeled for example with the hapten digoxigenin, which is then again bound by a further digoxigenin-specific antibody so as to allow for detection [0030]. This further antibody is itself labeled, for example with an enzyme such as peroxidase. Therefore, it would have been further obvious to employ such a further antibody in this manner so as to allow for detection of the results of the assay of MacDonald et al. and Karl et al.

With respect to claims 37-38, the immunoassay of Karl et al. involves binding antibody to a solid phase, such that the antigen is thereby captured on the solid phase during the assay [0026]. Such immunoassays may be characterized as an "immune binding assay" and may also be enzyme immunoassays in that they may use enzyme labels such as peroxidase [0030].

With respect to claim 60, McDonald et al. teaches detection of BNP in plasma as noted above. Karl et al. also teaches that all biological liquids known to the expert can be used as samples for the procedure of the method of identifying N-terminal proBNP. The samples preferred are body liquids like whole blood, blood serum, blood plasma, urine or saliva. The use

of blood serum and plasma is particularly preferred. See [0031]. Therefore, it would have been further obvious to employ the methods of MacDonald et al. and Karl et al. to analyze NT-proBNP canine serum rather than plasma, given that this type of sample was identified by Karl et al. as being particularly preferred for analysis of NT-proBNP.

Double Patenting

17. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

18. Claims 29, 31-38, 47, 57-58, and 60 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-4, 6-12 and 22 of copending Application No. 12/394,731. Although the conflicting claims are not identical, they are not patentably distinct from each other because the copending application also claims a method of detecting canine proBNP or a fragment thereof in a canine blood sample by contacting the sample with at least one antibody that binds to an epitope within SEQ ID NO:3 of canine

proBNP (see especially claims 1 and 7). Note that SEQ ID NO:3 is an epitope in "the Nt-proBNP region" as recited instantly, as evidenced by instant claim 31. The epitope may comprise at least 3 amino acids (see claims 2 and 14) and may be either monoclonal or polyclonal (claims 3-4). Detection may be via radioimmunoassay, immune binding assay, Western blot, immunohistochemistry, or enzyme immunoassay (see claim 6) and may employ peroxidase, biotin, fluorescent dye, gold colloid, or a radionuclide as labels for the antibody (see claim 16).

With respect to claim 47, although copending Application No. 12/394,731 fails to specifically recite a step of obtaining the antibody, it would have been obvious to do so as a necessary step before the antibody could be used in the recited method.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

19. Claims 29, 31-38, 47, 57-58, and 60 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-4, 6-12 and 22 of copending Application No. 12/394,682 in view of Harlow & Lane (Harlow, E. and Lane, D., Antibodies: A Laboratory Manual (1988) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pages 72-77, 555-561, 578-582, and 591-592; hereafter, "Harlow & Lane **3**"), Karl et al., and Liu et al.

Copending Application No. 12/394,682 recites a method of detecting canine proBNP or a fragment thereof in a canine sample comprising (a) providing a canine blood sample; (b) contacting the sample with at least one antibody that binds an epitope within SEQ ID NO:1; and (c) detecting the binding of the antibody to the epitope whereby the canine proBNP or the

fragment thereof in the sample is detected. See especially claim 1. Note that SEQ ID NO:1 is an epitope in "the Nt-proBNP region" as recited instantly, as evidenced by instant claim 31. The epitope may comprise at least 3 amino acids (see claims 2 and 14) and may be either monoclonal or polyclonal (claims 3-4). Detection may be via radioimmunoassay, immune binding assay, Western blot, immunohistochemistry, or enzyme immunoassay (see claim 6) and may employ peroxidase, biotin, fluorescent dye, gold colloid, or a radionuclide as labels for the antibody (see claim 16).

With regard to the elected species of an antibody binding to amino acids 32-48 of canine proBNP (SEQ ID NO:3), Copending Application No. 12/394,682 differs in that it recites the use of an antibody that binds an epitope within SEQ ID NO:1 (i.e., in the region from amino acids 1 to 22 of canine proBNP), rather than within SEQ ID NO:3 (i.e., within amino acids 32 to 48 of canine proBNP).

Harlow & Lane **3** is a laboratory manual for experiments involving antibodies. Harlow & Lane **3** teach that the most useful method to detect and quantitate antigens is the **two-antibody sandwich assay**, an immunoassay that is quick, accurate and reliable (pages 555, 559 and 579). This type of assay is conducted on a test solution and requires two antibodies that bind to non-overlapping epitopes, where one of the antibodies is bound to a solid phase and the other is labeled (see especially at pages 555-561, 578-582, and 591-592). Either two monoclonal antibodies that bind to discrete sites or alternatively one batch of affinity-purified polyclonal antibodies can be used to supply these two antibodies (page 579). To make the assay quantitative, results are compared to a standard curve obtained using known amounts of pure antigen (page 582).

Harlow & Lane **3** also provide extensive guidance with regard to how to obtain antibodies that are specific for a particular region of a protein using synthetic peptides (see pages 72-77, and especially at page 73, last paragraph). Further, Harlow & Lane **3** teach that when choosing such a peptide sequence, in general peptides of approximately 10 residues should be used as the lower limit for coupling; while peptides over 20 residues in length are increasingly difficult to synthesize.

In summary, it would have been obvious to one of ordinary skill in the art to employ an additional canine proBNP antibody in the method of copending Application No. 12/394,682 in order to allow for a two-antibody sandwich assay for canine proBNP to be performed. One would be motivated to do this because Harlow & Lane **3** taught that such sandwich assay formats are the most powerful of the immunoassays.

The teachings of Karl et al. and Liu et al. are discussed in detail above. Based on the findings of Karl et al. that amino acids 30-38 of human proBNP (ELQVEQTSL) was one of two epitopes which showed the strongest reaction with the polyclonal antibodies they elicited [0084], as well as the strong sequence and structural similarity between human and canine proBNP taught by Liu et al., it would also be reasonably expected that polyclonal antibodies raised against canine NT-proBNP would also be reactive with the canine NT-proBNP epitope corresponding to amino acids 30-38 of human NT-proBNP.

As such, armed with the knowledge that amino acids 30-38 of human NT-proBNP are highly immunogenic, as well as the detailed sequence information available that these amino acids correspond to amino acids 37-45 of canine NT-proBNP and that these amino acids are largely conserved across the two species, it would have been obvious to one of ordinary skill in

the art to target these analogous amino acids in canine NT-proBNP as they would have been reasonably expected to be immunogenic.

Further, when making an antibody against amino acids 37-45 of canine NT-proBNP, the resulting antibody would necessarily bind to amino acids 32-48 of this peptide since the former sequence lies entirely within the latter.

In summary, when the teachings of Karl et al. and Liu et al. are taken together with the guidance of Harlow & Lane that the two antibodies used for such assays must be *non-overlapping*, as well as the guidance that the antibodies should be raised using peptides that are generally at least 10 residues and no more than 20 residues in length, it would have been further obvious to select an antibody that bound within SEQ ID NO:3 as claimed.

This is a provisional obviousness-type double patenting rejection.

Response to Arguments

20. Applicant's arguments filed 12/20/2011 have been fully considered.

21. With respect to the rejections under § 112, 1st paragraph as failing to comply with the written description requirement, (Applicant's arguments (Reply, section C) have been fully considered but are not found persuasive.

Applicant argues that the Boswood Declaration establishes that it was not known whether the N-terminal region of proBNP (i.e., NT-proBNP) was present in detectable quantities in canine blood. Applicant argues that the present specification answered that question, in that the working examples demonstrated that the N-terminal region of proBNP was detectable in canine blood using antibodies.

Applicant also points to the instantly-filed Declaration under 37 CFR 1.132 by Dr. Farace ("Second Farace Declaration"), and argues this provides evidence that an antibody raised against SEQ ID NO:3 can successfully recognize and bind to canine proBNP in plasma.

The Declaration under 37 CFR 1.132 filed 12/20/2011 is insufficient to overcome the rejection of claims 29, 31-38, 47, 57-58, and 60 based upon 35 U.S.C. 112, 1st paragraph as set forth in the last Office action because:

Written description is evaluated at the time of filing. The post-filing experiments reported in the Second Farace Declaration are therefore not relevant to the inquiry as to whether Applicant has conveyed with reasonable clarity to those skilled in the art that, **as of the filing date sought**, he or she was in possession of the invention.

Moreover, Applicant's arguments are not persuasive as the Boswood Declaration does not merely state that it was not known whether the N-terminal region of proBNP (i.e., NT-proBNP) was present in detectable quantities in canine blood. Rather, the Boswood Declaration states that "it was not possible to predict that an antibody against a particular amino acid region of proBNP could detect proBNP or a particular N-terminal fragment of proBNP" (item 5).

The Boswood Declaration further states that "it was unclear how many forms of BNP existed in tissues and in blood" and that "it was unclear which amino acid regions of the various forms of BNP could be detected using antibodies" (Boswood Declaration, see item 7). Similarly, the Boswood Declaration states that "it remained unknown, in September 2004, how many forms of BNP are present in other species, such as cats and dogs, and which of those BNP forms would predominate in the plasma in those species" (item 8, see also at items 14 and 19).

Such statements therefore do not merely suggest that it was unknown whether NT-proBNP could be detected in canine blood; but rather, suggest that it was unknown whether this or any particular N-terminal fragment of proBNP existed in blood. Furthermore, the statements by Dr. Boswood that "it was not possible to predict that an antibody against a particular amino acid region of proBNP could detect proBNP or **a particular N-terminal fragment of proBNP**" suggests that one skilled in the art could not have predicted which N-terminal fragments of proBNP might be present, or that one skilled in the art could not have predicted that an antibody against SEQ ID NO:3 could detect proBNP or a particular N-terminal fragment of proBNP (such as NT-proBNP).

For these reasons, the examiner disagrees with the arguments by counsel regarding the Boswood Declaration. If one adopts the statements by Dr. Boswood, it is maintained that the specification's exemplification of antibodies binding to SEQ ID NOs 2 and 5 would not allow one to infer the existence of N-terminal fragments comprising SEQ ID NO:3.

In particular, the specification does not specifically identify what molecules reacted with the antibodies binding to SEQ ID NOs 2 and 5. For example, the specification does not disclose the molecular weight(s) or sequences of the molecules in canine blood that were captured by the antibodies.

If it was indeed not known how many or what forms of BNP are present in canine blood, and if it could not be predicted that an antibody against a particular region could detect proBNP or a particular N-terminal fragment of BNP, the disclosed experiments would only serve to verify the existence of molecules that contain SEQ ID NOs 2 and 5. If it was indeed unpredictable what particular forms or fragments of BNP molecules would circulate in canine

samples, one would still not know as a result of the above experiments whether N-terminal fragments containing SEQ ID NO:3 also existed, since antibodies binding to this epitope were not used to analyze canine samples. Similarly, if one skilled in the art could not predict whether an antibody against a particular region could detect proBNP or a particular N-terminal fragment of BNP, it would still remain unknown whether an antibody against SEQ ID NO:3 could detect proBNP or a particular N-terminal fragment of BNP (such as NT-proBNP).

22. Applicant's arguments with respect to the rejections under § 112, 2nd paragraph (Reply, section D) have been considered but are moot because the arguments do not apply to any of the references being used in the current rejection.

23. With respect to the rejections under § 103 based upon MacDonald et al. in view of Asada et al., Applicant's arguments (Reply, section E 1.) have been fully considered but are not found persuasive.

Applicant argues that one of ordinary skill in the art would have had no reasonable expectation of success because it was not previously known whether the N-terminal region of canine proBNP circulated in blood in amounts sufficiently abundant and/or stable to be detected by immunoassay.

In support of these arguments, Applicant points to a non-publication by Thomas et al. in which it is stated that BNP differs across species and which refers to species-specific variations likely to affect the BNP metabolism of BNP and also to different half-lives of BNP in different species. Applicant also points to Goetze et al. 2004 as teaching that the cleavage site for human BNP is not well-conserved across species. Applicant argues that these publications indicated the likelihood that BNP is metabolized differently across species.

This is not found persuasive for reasons of record.

It is initially noted that the claims refer to the detection of proBNP and/or NT-proBNP fragments thereof. The arguments that it was not previously known whether *NT-proBNP* could be detected by immunoassay are therefore not on point as this is not what is being claimed. In other words, the issue at hand is whether one of ordinary skill in the art would have had a reasonable expectation of success in finding either proBNP or amino-terminal fragments thereof (i.e., NT-proBNP).

In this regard, Applicant has argued on the record that it was known in the prior art that canine proBNP is cleaved into NT-proBNP and BNP, and that the NT-proBNP region of canine proBNP was also known (Applicant's arguments of 12/20/2011, see section D).

Furthermore, Asada teaches immunoassay methods for determining mammalian proBNP [0010]. The reference contemplates any mammalian proBNP and also specifically mentions canine proBNP (see [0010] and claim 1). Asada further teach that mammalian proBNP, a term which they indicate includes canine proBNP, can be detected in blood (ibid).

Asada therefore taught detection of mammalian proBNP in blood, including canine proBNP (see [0010] and claim 1). There is nothing of record that would clearly cast doubt on this teaching.

Moreover, the statements made by Asada that in blood, BNP exists in the form of proBNP or its degradation product, and not in the form of BNP-32 which was previously considered dominant [0008], are made generally and are not qualified as referring only to human BNP.

In addition, from the observation of canine BNP in blood (as taught by MacDonald), the existence of the larger proBNP precursor may be inferred. The existence of the mature peptide is strong evidence of the existence of its precursor. Similarly, as both the precursor proBNP as well as the C-terminal fragment thereof (BNP) were known to exist in dogs, it would have been reasonably expected that the N-terminal fragment of proBNP would also exist in blood.

Consequently, the evidence indicates that one skill in the art would have had a reasonable expectation of success in detecting not only circulating proBNP (as taught by Asada et al.), the circulating C-terminal fragment thereof (i.e., BNP, as taught by MacDonald et al.) and also the N-terminal thereof (i.e., NT-proBNP). Similarly, one of ordinary skill in the art would have had a reasonable expectation of success in detecting fragments containing SEQ ID NO:3 since this sequence is present both in proBNP and in NT-proBNP.

Regarding the Thomas et al. and Goetze et al. publications, it is noted that while Applicant relies on Thomas et al. as teaching different half-lives for BNP in different species and for teaching different metabolism, it is noted that Thomas et al. are here discussing the half-lives for administration of exogenous BNP as a drug. The reference is not discussing the half-life or metabolism of endogenously-produced BNP. As a result, this teaching does not call into question whether endogenous canine NT-proBNP would be expected to be found in blood or not.

Moreover, the fact that canine BNP differs from other species in sequence (as mentioned by Thomas et al.) fails to establish that one of ordinary skill in the art would lack a reasonable expectation of success in finding canine proBNP or NT-proBNP in blood, given the above teachings by Asada et al. and MacDonald et al., as well as Applicant's arguments that it was

known in the prior art that canine proBNP is cleaved into NT-proBNP and BNP, and that the NT-proBNP region of canine proBNP was also known.

Likewise, the mention by Goetze et al. that the cleavage site for human BNP is not well-conserved across species is not found persuasive to establish non-obviousness because the cleavage site for canine BNP was also known (Asada at [0010]).

Applicant has not adequately explained why such expected species-specific differences would mean that the ordinary artisan would doubt that proBNP or NT-proBNP could be detected in canine blood.

Similarly, Applicant argues while that MacDonald reports that plasma BNP increases with age in humans, this is not true for dogs (Reply, page 11, first paragraph). However, such observations are seen as tangential to the claimed assay method (which do not require measurement of proBNP as a function of age) and fail to establish that one of ordinary skill in the art would lack a reasonable expectation of success in detecting proBNP or NT-proBNP in canine blood.

Even if the ordinary artisan might expect BNP/proBNP/NT-proBNP to exhibit some differences across species, there is no evidence of record to suggest that such expected differences would rise to the level of not expecting these molecules to be detectable at all in blood.

Applicant's arguments that MacDonald relates to BNP-32 but does not provide any data or discussion of NT-proBNP in canines (Reply, page 11, second paragraph) and that Harlow & Lane, Wolfe and Janeway do not provide any specific guidance for making antibodies to canine

proBNP (Reply, paragraph bridging pages 11-12) are not found persuasive as they amount to a piecemeal analysis of the references.

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

In response to applicant's argument that MacDonald fails to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., a diagnostic test to identify patients with heart disease; see Reply, page 11, second paragraph) are not recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993). Similarly, Applicant's arguments that the ordinary artisan would lack a reasonable expectation of success in correlating the amount of protein detected with heart disease (Reply, paragraph bridging pages 11-12) are not found persuasive because no corresponding limitations are recited in the claims.

Regarding the statement by MacDonald that further studies are needed to verify their results (see Applicant's arguments at page 11, second paragraph), such statements are typical of those normally found in the non-patent literature and are not seen as sufficient to establish that the ordinary artisan would have lacked a reasonable expectation of success in arriving at the claimed invention.

Applicant's arguments that Asada does not teach whether the N-terminal region of proBNP exists in a stable and detectable form in canine blood (Reply, page 11, third paragraph)

are not on point as the claims are directed to the detection of proBNP and/or NT-proBNP fragments thereof. As above, Asada teaches detection of any mammalian proBNP and specifically mentions canine.

24. Applicant does not separately argue the limitations of dependent claims 34-36 or 60 (see Reply, pages 12-13).

25. With respect to the rejections under § 103 based upon MacDonald et al. in view of Karl et al. and Liu et al., Applicant's arguments (Reply, section E 4.) have been fully considered but are not found persuasive.

Applicant argues as above that MacDonald suggests but does not actually claim to provide a diagnostic test to identify patients with heart disease (Reply, page 13). This is not found persuasive because the features upon which applicant relies are not recited in the rejected claim(s).

Applicant argues as above that MacDonald does not provide any data or discussion of NT-proBNP in canines (Reply, pages 13 and 16), which is not found persuasive because as above (1) the claims do not require detection of NT-proBNP and (2) Applicant's arguments amount to a piecemeal analysis of the reference teachings.

Applicant also argues as above that the ordinary artisan would not have had a reasonable expectation of success. In support of this position, Applicant points to the teachings of Liu which establish that the sequences of human BNP and canine proBNP are different. Applicant also points to the teachings of Liu which states that antibodies of BNP are species-specific, to the teachings of Thomas et al. suggesting that BNP does not have strong homology across species,

and to the first Farace Declaration as evidence that antibodies to canine NT-proBNP do not recognize human or cat NT-proBNP. See Reply, pages 14-15.

Applicant further argues as above that the ordinary artisan would not have had a reasonable expectation of success in detecting canine proBNP, or a fragment thereof containing SEQ ID NO:3, in a canine blood sample pointing to the teachings of Thomas et al. and MacDonald et al. which discuss differences in BNP across species. See Reply, pages 15-16.

This is not found persuasive because although the amino acid sequences of BNPs across species may differ, at the time of the invention the sequence of canine BNP was known in the art. It was also well known in the art how to raise antibodies against a known amino acid sequence.

As illustration of this common knowledge in the art, Liu et al. reported the newly identified cat BNP sequence and discuss how this information now allows for antibodies to be generated using synthesized antigenic peptides for immunological assay development, and in particular for clinical applications (paragraph bridging pages 188-189).

Similarly, Luchner et al. (Am J Physiol Heart Circ Physiol 274:1684-1689, 1998, of record) concisely state how they employed a polyclonal antibody specific for canine BNP in order to measure BNP in dogs, because of the known species variability in BNP (see page 1685, "Analytical methods").

This provides evidence that species-specific differences were known but that rather than presenting an insurmountable technical obstacle, the ordinary artisan was familiar with how to deal with this issue; namely to use species-specific antibodies.

Similarly, the evidence of record indicates that notwithstanding the known differences across species, BNP was nonetheless thought to share related functions in mammalian species. See for example Liu et al. at page 188, right column, first full paragraph, last sentence.

In summary, even if the ordinary artisan might expect BNP/proBNP/NT-proBNP to exhibit some differences across species, there is no evidence of record to suggest that such expected differences would rise to the level of not expecting these molecules to be detectable at all in blood.

With respect to Applicant's arguments that Karl reports only that *human* BNP is more stable than BNP-32, but does not discuss the stability of *canine* BNP or BNP-32 (see Reply, page 16), it is noted that Karl discusses the stability of BNP in general and does not qualify these remarks in terms of *human* BNP alone. Rather, in one passage in which Karl et al. discuss the stability of BNP, the reference explicitly mentions both human and pig BNP [0006]. Consequently, it is maintained that one of ordinary skill in the art would *reasonably* expect the mature form of BNP to be less stable across species.

For all of these reasons, it is maintained that the evidence of obviousness fails to outweigh the evidence of non-obviousness.

26. With respect to the provisional obviousness-type double patenting rejections, Applicant argues that if a provisional ODP rejection is the only remaining rejection in one of the co-pending application that the rejection should be withdrawn (Reply, section F).

The rejections are maintained at this time for reasons of record as they are not the only remaining rejections.

Conclusion

27. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Christine Foster whose telephone number is (571) 272-8786. The examiner can normally be reached on M-F 8:30-5:00. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mark Shibuya, can be reached at (571) 272-0806. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Christine Foster/
Examiner, Art Unit 1641

/Mark L. Shibuya/
Supervisory Patent Examiner, Art Unit 1641